REMARKS

Claims 14, 23-25 and 32-37 presently appear in this case. No claims have been allowed. The official action of May 23, 2001, has now been carefully studied. Reconsideration and allowance are hereby respectfully urged.

Briefly, the present invention relates to monoclonal antibodies which are end-specific for the free N-terminus of an amyloid β-peptide or the free C terminus of the Aβ1-40 peptide or the Aβ1-43 peptide. The monoclonal antibodies of the present invention do not bind to the amyloid β-precursor protein from which the amyloid β-peptide may be proteolytically derived. The present invention further relates to single-chain antibodies which are similarly end-specific for either the free N-terminus or the free C-terminus of an amyloid β-peptide.

It is noted that the examiner has pointed out that the references in the specification are not a proper IDS and that references should be submitted on a form PTO-892 in order to be considered.

An IDS is now being prepared and will be submitted for the examiner's consideration within a short period of time.

It is noted that the examiner has indicated that the drawings submitted with this application have been approved by the draftsperson.

The examiner has withdrawn claims 1-13, 15-22 and 26-31 from further consideration as being drawn to an invention non-elected without traverse.

All of the non-elected claims have now been deleted without prejudice toward the continuation of prosecution thereof in a continuing application.

Claims 14 and 23-25 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The examiner states that the claims recite "end-specific for the terminus of an amyloid β-peptide (Aβ)" monoclonal or single-chain antibodies. However, the examiner states that Aβ-peptide refers to a heterogeneous population of amyloid peptides, wherein both the N- and C-terminals are heterogenous. For example, the examiner states that the art of record refers to both Aβ(1-40) and Aβ(1-42), which documents the heterogeneity at the C-terminus. The examiner states that the art of record also documents the heterogeneity of the N-terminus. Thus, the examiner states that, in the absence of a defined specific sequence epitope to which the antibody binds, the metes and bounds of "end-specific for the

N-terminus or the C-terminus of an amyloid β-peptide" cannot be ascertained. This rejection is respectfully traversed.

There are three main Aβ-peptides which are involved in the pathogenesis of Alzheimer's disease, Aβ1-40, Aβ1-42 and Aβ-143. See page 13, lines 18-24, of the present specification. All of these have a common N-terminus. They have a total of three different C-termini. The present invention relates to the use of antibodies that are amino- or carboxy- free-end specific for amyloid β-peptides as a method to selectively inhibit accumulation and/or neutralize the cytotoxicity associated with amyloid fibrils. The most effective target for free-end specific antibodies when used in this way as therapy for Alzheimer's disease, is therefore considered to be Aβ1-40, which forms the bulk of circulating amyloid β-peptide (human CSF, plasma, and urine), or the more toxic but less abundant Aβ1-42 and Aβ1-43 species that can seed amyloid deposition. Neuritic plaques and vascular amyloid deposits contain an abundance of these forms and the consensus that has evolved from genetic and biochemical analyses of human tissue and transgenic mouse models is that full-length forms of amyloid β-peptide are the key players in the pathogenesis of Alzheimer's disease. Removal of these major forms, or limiting their neurotoxicty, can therefore be expected to slow progression of Alzheimer's disease and delay

onset in susceptible individuals. This finite number of peptides is not indefinite.

Notwithstanding the importance of full length Aβ-peptides as major therapeutic targets, the invention also envisages using antibodies that are free-end specific for any other amyloid β peptide proteolytically-derived fragment that is neurotoxic and/or can form fibrillar deposits. As stated by the examiner, a number of studies have indicated heterogeneity at both the N-terminus and C-terminus of amyloid β peptides. For example, there is an appearance of N-terminus-truncated amyloid β-peptides in early, diffuse plaques of Alzheimer's disease. Yet the failure of diffuse plaques to evolve into neuritic plaques and the absence of N-terminus-truncated amyloid β-peptides in mature plaques strongly argues against their pathogenic significance. While recognizing the dominant role of full-length Aβ-peptides, the present invention is not limited to these forms. The present invention also teaches the production and use of free-end specific antibodies that specifically recognize N-terminus-truncated amyloid β-peptides species, but, importantly, not the amyloid precursor protein from which they were derived. The present specification states, at page 13, lines 24-31, that other Aβ-peptides having different beginnings or ends

sometimes exist and that the present invention encompasses free-end specific antibodies directed also to such peptides. This is not indefinite as those of ordinary skill in the art would well be able to determine the metes and bounds of the present specification. An antibody that is specific to the free end of any Aβ-peptide and which does not bind to the amyloid β-precursor protein from which such amyloid β-peptide may be proteolytically derived, is part of the present invention. As there are only a finite number of such Aβ-peptides, there are only a finite number of antibodies in accordance with the present invention. This fully complies with the second paragraph of U.S.C. §112. Reconsideration and withdrawal of this rejection are, therefore, respectfully urged.

Claim 14 has been rejected under 35 U.S.C. §102(b) as being anticipated by the European patent to Takeda, the Konig publication or the Tsuzuki publication. The examiner states that Takeda teaches monoclonal antibodies that are specific for the N-terminal and C-terminal of Aβ; that Konig teaches monoclonal antibodies that are specific for the N-terminal and C-terminal of Aβ; and that Tsuzuki teaches a monoclonal antibody that binds Aβ(1-9). This rejection is respectfully traversed.

It is essential to understand the distinction between "N-terminus specific", "C-terminus specific" and "end-specific". The first two terms are known in the prior art as meaning that the antibody is specific to an epitope somewhere near the N-terminus of the peptide or somewhere near the C-terminus of the peptide. However, the term "end-specific" is defined in the present specification, for example at page 10, lines 8-12, where it states:

> Such a recombinant antibody molecule discriminates between an Aβ peptide and the β-amyloid protein precursor from which it is proteolytically derived, and is also referred to throughout the present specification as an "antisenilin".

Does not bind APP

See also the definition at page 12, lines 29-33, where it states:

> [W]here an end-specific antibody is defined as an antibody which uniquely recognizes the free N-terminus or the free C-terminus of a peptide and which can further discriminate between the peptide and the precursor from which it is proteolytically derived.

APP

Reference is also made to Example 1 of the present specification relating to the strategy and protocols for use in developing such an antibody. In the section bridging pages 25 and 26 of the present specification, entitled "ELISA detection and affinity determinations", the spanning peptide ELISA detection method is described. In this technique, all antibodies that bind the sequence of amino acids in the

spanning peptide (SEQ ID NO:7), which spans the splice site of the precursor protein, are eliminated from the screen. Then antibodies that bind to a peptide of SEQ ID NO:6, having amino acids 1-6 of Aβ1-40, are selected. Note page 26, lines 5-13, which states:

> The same spanning peptide was coupled to a thiol coupling gel via their cysteine residue and used to preabsorb away all antibodies which do not depend upon the free amine-Asp being present. The antibodies were then purified and collected using the N-terminal peptide. Whereas the crude serum shows substantial activity toward the spanning peptide, once affinity purified, there is no reactivity of the resulting antibody with the spanning peptide, only with the N-terminal peptide.

These selected antibodies are now referred to in the claims as "free-end specific for the free N-terminus of an amyloid β-peptide" because their binding epitope incorporates both a high affinity recognition element for a free amino (NH_2) group, in addition to recognition of the N-terminal amino acid residues of the particular amyloid β-peptide. Furthermore, the claim now makes explicit the language from the definitions quoted above that the antibodies which are free-end specific for a free terminus of an amyloid β-peptide do not bind to the amyloid β-precursor protein from which said amyloid β-peptide may be proteolytically derived. A similar detection system may be used for selecting monoclonal antibodies that are C-end-specific using a spanning peptide that corresponds to a

contiguous sequence of amino acids on both of sides of the C-terminal cleavage sites in APP.

The importance of achieving free-end specificity cannot be over-emphasized. This exquisite sensitivity of free-end specific antibodies is required so as not to affect the normal biological functions of the transmembrane receptor-like APP molecule that is implicated in several important physiological roles. For example, it has been reported that the amyloid precursor protein (APP) mediates adhesion, has growth-promoting effects, is an inhibitor of serine proteases, and is important for neuroprotection, neuritic outgrowth, recycling of synaptic vesicles, regulation of apoptosis, receptor and signal transduction functions, calcium metabolism, and gene transcription. Thus, the present invention utilizes *free-end specific* antibodies to inhibit the accumulation of amyloid β-peptides and ameliorate the neurotoxic consequences of amyloid deposition without affecting the important physiological roles of APP.

Without the spanning peptide ELISA detection method described in the present specification, it would not be possible to select antibodies that are free-end specific for amyloid β-peptides since such antibodies are extremely rare. Note the above quote from page 26, where it states that the

crude serum shows substantial activity toward the spanning peptide.

The following example is very similar to that of Example 1 of the present specification and can be submitted in the form of a declaration if the examiner indicates that such would be useful.

Example: Peptide H_2N-Asp-Ala-Glu-Phe-Arg-aminohexanoate-Cys-amide was conjugated to BSA through a SMCC linker. Swiss Webster mice were immunized with 100 µg of this conjugate in Freund's complete adjuvant and then boosted twice with a further 100 µg of conjugate in Freund's incomplete adjuvant. Fusions were performed on spleens of reactive mice, and hybridomas screened using the "spanning peptide ELISA detection method", as described in the specification. Briefly, Aβ1-40 was coated onto 96-well plates, which were then incubated with samples in the presence of competing peptides A (immunogen), C (spanning peptide), or Aβ1-40 itself. The common result is shown in Figure 1 (attached hereto). As expected, antibodies produced by these animals bind residues 1-5 of Aβ (peptide A), which was the peptide used for immunization, and the full-length Aβ1-40 peptide. However, these antibodies are also reactive with the Aβ1-5 epitope when flanked by additional sequences on its N-terminus (peptide C), as is the case in the intact amyloid precursor

protein APP. Such antibodies may be referred to as epitope-specific (for sequences 1-5 of the N-terminus of Aβ).

A much more rare result seen upon testing of samples is shown in Figure 2, attached hereto. These antibodies bind the immunizing peptide (Aβ1-5) and the Aβ1-40 sequence, but do not recognize the peptide derived from the region which spans the same 5 amino acid sequence in the full length APP (peptide C). The exquisite selectivity of these antibodies lies in their ability to bind the 1-5 sequence only when positioned at the free N-terminus of the molecule; these antibodies are referred to as *free-end specific*.

The Takeda specification teaches monoclonal antibodies that are specific for N-terminal portions of amyloid β-peptides. These monoclonal antibodies are not *free-end* specific as defined herein. Thus, antibodies BAN-052a and BAN-50a are described as "reactive to the partial peptides on the N-terminal sides of the β-amyloids or the derivatives thereof"; as examples, these antibodies are said to recognize the partial peptides having the sequences corresponding to Aβ1-28 and/or Aβ1-16. There is no requirement here for a free N-terminus specific binding epitope. Moreover, the 16 and 26 amino acid residue peptide antigens used would be expected to present a multitude of binding epitopes. Importantly, the Takeda specification does not describe a method for selecting

antibodies that specifically bind to the free amino terminus of amyloid β-peptide but would not bind the same sequence in APP. Reconsideration and withdrawal of this part of the rejection are, therefore, respectfully urged.

Konig et al teach an antibody (Mab 286.8A) that is specific for sequences in the N-terminal region of Aβ, and the use of this antibody for the detection of Aβ species *in vitro*. MAb 286.8A was shown to be specific for an epitope comprising residues 3-8 of the amyloid β-peptide. There is no suggestion that MAb 286.8A would not bind the identical sequence of amino acids in the intact APP protein. Moreover, MAb 286.8A was not selected using a detection system that can specifically differentiate antibodies that bind the free N-terminus. Since free-end specific antibodies are usually very rare within a given population of hybridomas, and in view of the disclosure that the binding epitope does not even include residue 1, it is apparent that MAb 286.8A is not end-specific as defined herein. Reconsideration and withdrawal of this part of the rejection are, therefore, also respectfully urged.

In Tsuzuki, the authors state that monoclonal antibody Aβ90/12 "reacts with the Aβ N-terminus, and thus reacts with APP and other APP cleavage products ...". By definition Aβ90/12 is therefore not free-end specific as defined herein and thus does not anticipate claim 14.

Reconsideration and withdrawal of this part of the rejection are, therefore, also respectfully urged.

Claims 14, 23, and 25 have been rejected under 35 U.S.C. §102(e) as being anticipated by Solomon. The examiner states that Solomon teaches a monoclonal antibody AMY-33 that is specific for the N-terminal amino acids 1-28 of Aβ and that Solomon teaches single-chain antibodies based thereon and their use as therapeutic chaperones for the treatment of Alzheimer's Disease. This rejection is respectfully traversed.

AMY-33 is specific for a contiguous sequence within the N-terminal region of Aβ, but is not free-end specific as defined herein. This antibody was generated by immunization with a peptide corresponding to sequences 1-28 of Aβ. Those skilled in the art would understand that a peptide antigen of 28 amino acids would give rise to a multitude of epitopes of which very few would be likely to incorporate the free amino group at the N-terminus. The selection of AMY-33 did not include a detection method such as that described by the present invention to select the rare free-end specific epitope. On the contrary, the selection of AMY-33 was based on its ability to inhibit the aggregation of the amyloid β-peptide (Solomon et al. 1996 PNAS 93:452-455). Importantly, it has been demonstrated that the critical epitope with

respect to anti-aggregating activity is localized within the sequence Glu-Phe-Arg-His at positions 3-6 of amyloid β-peptide (Frenkel et al 1998 J Neuroimmunology 88:85-90; Frenkel et al 1999 J Neuroimmunology 95:136-142). These antibodies are clearly not end-specific as defined herein because they do not specifically bind the free amino group of the N-terminus Asp at position 1. As Solomon does not teach any free-end specific antibody, it cannot teach any single-chain antibody with the same specificity. Therefore, the teaching of single-chain antibodies based on the AMY-33 antibody is not relevant to the current claims. Furthermore, claims 25 relates to C-terminal free-end specific antibodies and is, therefore, not relevant in the context of the Solomon specification, which deals only with antibodies that bind in a region close to the N-terminus. Accordingly, reconsideration and withdrawal of this rejection are respectfully urged.

Claims 14 and 23-25 have been rejected under 35 U.S.C. §103(a) as being unpatentable over any one of Takeda, Konig or Tsuzuki in view of Seubert and Duenas. The examiner states that Takeda, Konig and Tsuzuki teach end-specific antibodies but not single-chain antibodies. The examiner states that Seubert teaches that the use of antibodies that bind Aβ-peptides are useful in the diagnosis of probable Alzheimer's Disease and are useful for detecting Aβ-peptides

in vitro or *in vivo*. The examiner states that Seubert also teaches the use of antibody fragments for this purpose and recombinantly-produced antibodies. The examiner states that Duenas teaches conventional methods of expression of a single-chain Fv antibody fragment in *E. coli* and that the production of such single-chain antibodies was conventional at the time of the present invention. Accordingly, the examiner considers it to have been *prima facie* obvious to modify the end-specific monoclonal antibodies of Takeda, Konig and Tsuzuki by means of expression as a single-chain Fv antibody fragment according to the vectors and methodologies of Duenas because Seubert teaches that Fv and other antibody fragments, including those that have been recombinantly produced, that bind Aβ-peptides are useful in a variety of detection techniques for use in screening or diagnostic assays. This rejection is respectfully traversed.

First of all, as detailed above, none of the antibodies described in these citations are N-terminal free-end specific as defined herein. Therefore, it would not have been obvious at the time of the present invention to make single-chain antibodies that are free amino terminus end-specific for amyloid β-peptide. With respect to C-terminal antibodies, the present claims also are specifically directed to free-end specific antibodies to the free C-terminal end of

Aβ. None of the prior art described methods for selecting antibodies that are C-terminal free-end specific for amyloid β-peptides. However, one such monoclonal C-terminal specific antibody for Aβ1-42 was shown not to recognize Aβ1-43 (MAb 369.2B of Konig). This antibody was found fortuitously, and there is no indication of its advantage. Thus, it would not be obvious to seek monoclonal antibodies which specifically bind to the free carboxy group at the C-terminus of amyloid β-peptides Aβ1-40 or Aβ1-43, nor is there any technique disclosed for reproducibly obtaining such antibodies. Accordingly, new claims 33 and 34 have now been added specifying that the antibody is free-end specific for the free C-terminus for the amyloid β-peptide Aβ1-40 and Aβ1-43, respectively. Claims 36 and 37 are directed to single-chain antibodies with the same specificity.

As discussed above, none of Takeda, Konig or Tsuzuki disclose free-end specific antibodies directed to the free N-terminal of an Aβ-peptide, and specifically Aβ1-40, Aβ1-42 or Aβ1-43. Accordingly, claims 14, 32, 24 and 35 cannot be obvious over any combination of the references, particularly since Seubert and Duenas also do not teach any such N-terminal peptides. Similarly, claims 33, 34, 36 and 37 cannot be obvious from the combination of references as none of the references disclose any kind of antibody that is free-end

specific for the free C-terminus of amyloid β-peptides Aβ1-40 or Aβ1-43. The prior art provides no motivation to seek out such antibodies. Accordingly, these claims must also be unobvious from the references of record.

With respect to claims 23 and 25, the only antibody which is fortuitously free-end specific for the free C-terminus of an amyloid β protein is specific to the Aβ1-42 peptide. This is MAb 369.2B. This antibody recognizes the C-terminus Aβ species ending in position 42. It does not recognize the shorter Aβ species 1-40 in solution or in solid phase. This publication clearly states that the antibody was identified and characterized using three criteria: (1) immunoprecipitation of in vitro translated Aβ1-42; (2) as in (1) but with an excess of competitor peptides Aβ1-40, 1-11, 1-28, and 12-28; (3) solid phase ELISA to detect recognition of peptides Aβ35-42 and Aβ1-42 in the presence of competitor peptides Aβ35-42, 1-42, and 1-40. The selection of MAb 369.2B, therefore, did not include a detection method such as that described by the present invention to select a rare free-end specific antibody. However, upon further characterization of MAb 369.2B in immunocytochemical studies, it was demonstrated that tissue staining was competed out with Aβ-peptides 35-42 and 1-42, but not with Aβ1-43. Therefore, this

antibody, while not selected as such, appears to have free-end specificity for the peptide Aβ1-42.

A single chain antibody having this specificity is not anticipated, as is recognized by the Examiner. The Examiner must rely on obviousness to attempt to render such a single chain antibody unpatentable. However, the single chain antibody specific to the C-Terminus of Aβ1-42 has properties which are not recognized by Konig, Duenas, or Seubert which properties rebut any *prima facie* case of obviousness established by the Examiner. There is no recognition in Konig that this antibody will have any properties any different from any of the other antibodies of Konig. However, the present specification teaches that such an antibody has tremendous advantage for the purposes of the present invention in that it will not bind to the intact APP, and thus will not effect the various beneficial properties of APP while attacking the Aβ-peptide which forms the plaque which causes Alzheimer's disease. Accordingly, claims 23 and 25 are also patentable for the reasons discussed herein. Reconsideration and withdrawal of this rejection are therefore respectfully urged.

It is submitted that all of the claims now present in the case clearly define over the references and fully comply with 35 U.S.C. § 112. Reconsideration and allowance are therefore earnestly solicited.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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Version with Markings to Show Changes Made

Claims 14 and 23-25 have been amended as follows:

14 (Amended). A monoclonal ~~Monoclonal~~ antibody which is free--end- specific for the free N-terminus of an amyloid β-peptide, which antibody does not bind to the amyloid β-precursor protein from which said amyloid β-peptide may be proteolytically derived.

23 (Amended). A single chain antibody ~~having~~ which is free-end- specific ~~Aβ binding capability~~ for the free N-terminus or free C-terminus of an amyloid β-peptide, which antibody does not bind to the amyloid β-precursor protein from which said amyloid β-peptide may be proteolytically derived.

24 (Amended). A single chain antibody in accordance with claim 23, which is free-end- specific for the free N-terminus of an amyloid ~~β~~ β-peptide.

25 (Amended). A single chain antibody in accordance with claim 23, which is free-end- specific for the free C-terminus of an amyloid ~~β~~ β-peptide.

New claims 32-37 have been added.

Claims 1-13, 15-22 and 26-31 have been deleted.